STEM CELLS FOR B CELL REPLACEMENT, VALERIA SORDI

State-of-art and future development/perspectives of the research area at international level (max 2000 characters):

CELL THERAPY FOR DIABETES. Type 1 diabetes can be treated with β cell mass replacement therapy. Decades of donor islet transplant experience have taught us a lot about the ability of β cells to engraft and function upon transplantation. Islet transplantation, however, is severely limited by the scarcity of organ donors and the need for lifelong immunosuppression. All the knowledge generated is now invested in the great challenge of regenerative medicine.
NEW SOURCES OF BETA CELLS. Nowadays it is in fact possible to generate functional β cells from the differentiation of pluripotent stem cells, both embryonic and induced pluripotent stem cells (iPSC), which can be genetically reprogrammed from somatic cells of each individual. Differentiation protocols are established, although constantly improving.
STEM CELLS AND CLINICAL TRIALS. The first pioneering clinical trials with these cells started in the US and are ongoing. Viacyte experience proves that stem cell-derived β cells can engraft and secrete insulin in patients with T1D, but that cell maturation and survival need to be improved. Clinical experience of infusing mature stem cell-derived β cells in the portal vein by Vertex sounds highly promising but at a very early stage of testing. New big consortia are being formed also in Europe thanks to a collaboration between pharma and academia.
FUTURE DEVELOPMENTS. The three main current challenges for the newly generated stem cell-derived β cells are quality, safety, and immune escape. The field will expand to embrace a wider pool of recipients: not only T1D but also T2D, "genetic" diabetes, pancreatectomized subjects. In the future autologous personalized cell product will be produced.

Actual lines of research (as is) of the Diabetes Research Institute (max 2000 characters):

Stem cells for β cell replacement research area is mainly focused on iPSC-derived β cells (iβ) and can be divided into 3 sub-areas:

1) iβ for clinical application
- differentiation of iPSC into β cells: scale-up (2D>3D)
- generation of GMP iPSC line and GMP diff protocol (in collaboration with Policlinico di Milano, Plagencell grant by Regione Lombardia)
- automation and high throughput production of iPSC lines and iβ (in collaboration with OSR-CRB)
2) iβ improvement
- differentiation of iPSC into β cells: improve efficiency (% of mono-hormonal insulin-positive cells and ability to secrete insulin in response to stimuli)
- identification of gene signature of endoderm-committed iPSC DRI1 clones
- in vivo models of iβ transplantation (kidney, liver, w/wo diabetes, in decell lung, in collaboration with Antonio Citro)
- development and optimization of the micro-organ composed of decell lung micro-scaffold and iβ (in collaboration with Betalin)
- depletion of stem cell contaminant of iβ for safety issue.
- Differentiation of “stealth” iPSC into iβ for immune escape studies (in collaboration with Raniero Chimienti and Silvia Torchio).
3) iβ for cell therapy of “genetic” diabetes
- Wolfram iPSC > iβ
- MODY iPSC > iβ

DRI is also part of the Vertex clinical trial VX-880 (site accreditation and patient recruitment ongoing, in collaboration with OSR-MITRA). The trial consists of intraportal transplantation of embryonic stem cell-derived mature β cells, in patients with T1D and the same clinical indications of islet transplantation.

Strengths of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

OSR patients (T1D, MODY and other forms of “genetic” diabetes) and facilities (iPSC Hamilton facility, animal-imaging-genomics facilities)

DRI has a long, well recognized, expertise in islet transplantation (islet culture, portal vein infusion, selection of recipients, immunosuppressive therapy). PIP provides islets and pancreatic material for research studies.

DRI has a strong recognized expertise in animal model of β cell replacement (different sites, metabolic tests, diabetes management)

DRI is part of prestigious national and international consortia that make data and info available on a very large network of collaborators (Plagencell, H2020 Brussels, Espace, and Vanguard).

DRI biobank (potentially expandable to iPSC banking)

The stem cell group at DRI has 10 years of experience in iPSC diff into β cells. Team is qualified and motivated.

β cell replacement research area is well recognized at national level.

Strong fundraising potential.

Weaknesses of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

For cell therapy with autologous iPSC-derived β cell the main weaknesses are time/cost of personalized cells and the safety issues intrinsic to autologous therapy. For cell therapy with allogeneic iPSC-derived β cell the main weakness is the potential risk introduced by cell genome modification. For both approaches, GMP iPSC lines and GMP protocols for differentiation and gene editing, set up in dedicated cell factories, are required, which implies strong collaboration with outside or internal investment.
Other weaknesses:
The research area is moderately/scarcely recognized at european/world level.
Lack of strategic expertise (gene editing, genetics, β cell biology).
Poor collaboration with clinicians (no easy access to data and samples). The research area relies on collaborations with uncertain future (Policlinico Cell Factory, iPSC OSR facility).

Lack of staff training: basic scientific knowledge in the diabetes field (young people, temporary staff), project and people management (senior staff), IT updating (all).
Lack of cell factory (need to look outside, to public and private cell manufacturing companies).
Frequent turnover of people with technical competencies>> lack of technicians/lab managers in charge of bureaucracy, standard lab protocols, safety, animal experimentation, research quality.

Short-medium term OSR/UniSR goals (0-18 months): milestones and deliverables (max 1000 characters):

In 2021/22 we will start working with GMP iPSC line, contribute to the start-up of the iPSC facility, carry on the projects on (i) iβ safety (Brentuximab), (ii) selection of best iPSC clone and (iii) in vivo function of iβ.

Milestones
M1) drafting of a GMP-compatible 3D protocol
M2) set up of iPSC reprogramming at the iPSC Hamilton facility
M3) test differentiation capacity of new iPSC line from Policlinico
M4) validation gene signature of endoderm-committed clones on other iPSC clones
M5) definition of the best tx site in mouse models (liver vs kidney)
M6) determination of the best schedule of treatment of iβ with Brentuximab for complete PSC depletion.

Deliverables
D1) new GMP 3D diff protocol
D2) 1 iPSC clone reprogrammed at the facility
D3) selection of best clone for GMP production at Policlinico
D4) best in vivo model of iβ tx
D5) gene signature of endoderm-committed clones
D6) protocol of treatment of iβ with Brentuximab

Medium term OSR/UniSR goals (18-36 months): milestones and deliverables (max 1000 characters):

In 2022/24 we will continue producing iβ (at DRI and at the facility, research-grade and GMP, from control and diabetic subjects) and we will test strategies for safe and immune-evasive iβ tx. If Vertex supports it, we will also plan an analysis of VX-880 recipients.

M7) Establishment of an iβ Foundry: a subgroup of people dedicated to iPSC reprogramming, culture and differentiation into β cells. The Foundry will guarantee the production of cells on a continuous basis, with high quality and reproducibility.
M8) final evaluation of GMP iPSC line with Policlinico
M9) validation of protocol obtained in D1 on GMP iPSC.
M10) set up of iPSC diff into β cells at the facility
M11) reprogramming of iPSC from patients with diabetes (T1, T2, genetic)
M12) generation of iPSC with suicide gene system
M13) proof of immune escape of stealth iβ in vivo

D7) provision of iPSC lines and iβ for scientific projects, inside and outside DRI
D8) GMP iPSC
D9) iPSC lines from patients with diabetes

Long term OSR/UniSR goals (36-60 months): milestones and deliverables (max 1000 characters):

In 2024/25 we will produce GMP iβ (in collaboration with Policlinico or other cell factories), and bring the best iβ in terms of safety and immune escape to the next step for clinical application. We will also have a high throughput production of iβ research grade in the iβ Foundry or at the iPSC Hamilton facility. If Vertex trial moves to phase 2, we will follow recruitment, transplantation and follow-up of recipients.

M14) preclinical testing of GMP iβ
M15) validation of safety switch in in vivo models

D10) GMP iβ
D11) high throughput iβ differentiation at the iPSC Hamilton facility
D12) iβ Foundry becomes a facility (self-supportive)
D13) iPSC and iβ with safety switch
D14) in vivo stealth iβ
D15) IMPD of iβ (autologous/stealth, with suicide gene/Bre treatment)

Investments of the Diabetes Research Institute (e.g. personnel, space, technology) to achieve the short-medium-long term goals (max 2000 characters):

Personnel: the group needs PI (VS), 3 post-docs, 1 PhD student, 1 technician (stable!!!), 1/2 undergrad students (link with UNISR).
Shared with other groups:
- 1 secretary (orders, missions, contracts),
- 1 grant manager (grant application, activation and reporting, organization of meetings: lab meeting, congresses, fundraising events),
- 1 communication manager (DRI internal and external comm: newsletter, branding, website and social media, in collaboration with OSR/UniSR communication staff),
- Technicians/lab managers in charge of
* bureaucracy (new people at DRI, mailing list, lab and desk spaces),
* standard lab protocols (liquid N2, cell culture, biomol)
* safety (training, control, maintenance)
* animal experimentation (IACUC, access to animal facility, protocols, reagents, surgical instruments)
* research quality assessment

Space: 1 lab (also shared with others, 3 benches could be enough), 1 cell room DEDICATED TO IPSC and with access restricted to qualified personnel, adequate (?) equipped space in the animal facility, 1 work station at CRB close to the iPSC facility, space dedicated to molecular biology (shared with others, with pre/post PCR spaces). Six desks, at least 3 PCs for bioinfo and image analysis.

Technology:
If iPSC facility works as hoped, we will use it for automated high throughput manufacturing of iPSC and iβ. If iPSC facility is not available, we will need an automated system for iPSC reprogramming and differentiation.
The clinical iβ project needs a cell factory for GMP production of iβ (internal, or strong link with external)

DRI would benefit from the presence of a scientific advisory board (2-3 international scientists in the field) that every 3 years analyzes strategic lines of research/milestones and deliverables/people careers.